Structure of the Vegetative Shoot Apex of *Clethra barbinervis*

III. Longisectional View, Summary Analysis and Discussion*

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The vegetative shoot apex of *Clethra barbinervis* Sieb. et Zucc. was analyzed in longisectional view and discussed from the viewpoint of the three-dimensional aspect.

The width of the central zone fluctuates during a plastochron but the depth scarcely changes. The beginning of leaf initiation is recognized in a very early stage of growth even in longisectional view, if sector development is carefully examined.

When two reconstructed and overlapped figures for the first cell layer of the apex are depicted, changes occurring at the apex in the superficial view come to be easily understood. Moreover, if the shoot apex is analyzed for T divisions, a type of analysis recommended for the root apex by Schüëpp (1917), anatomical changes of the apex are fairly well understood even from the viewpoint of the three-dimensional aspect.

Superficial and transectional views of the vegetative shoot apex of *Clethra barbinervis* Sieb. et Zucc. were analyzed in earlier papers of the present series (Hara, 1971a, b). In the present study the apex of the same species is analyzed in longisectional view, and a summary analysis and discussion of the three-dimensional construction of the apex are given.

Materials and Methods

The material was collected at the same time and treated in the same way as described previously (Hara, 1971a, b). Approximately 20 apices collected at Nippara were sectioned longitudinally. Photographs are of these sections. In addition, many vegetative and reproductive shoot apices, collected mainly at Nikko, were sectioned and observed. The transectional material used is the same as in the former papers (Hara, 1971a, b).

Results — Longisectional View

Outline: The outline of the vegetative shoot apex fluctuates from flat to slightly convex. The flat area at the apex is rather large in the maximal area phase of a plastochron and becomes small in the minimal area phase (Figs. 2–10 in Hara, 1971a;

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Figs. 1-4). The diameters of the apex were already reported; the longest diameter measured is $339\ \mu$ and the shortest, $78\ \mu$ (cf., Hara, 1971b).

Arrangement of tunica and corpus: It is convenient to describe the organization of the apical meristem by the tunica-corporis concept which is based on the plane of cell divisions. I prefer a flexible use of the tunica-corporis terminology in determining the number of superficial layers of the apex which fluctuates in various species. The number of the tunica layers in the apex of *Clethra barbinervis* is judged to be one in most instances (Figs. 1-4), as already reported by Hara (1958). Sometimes the second cell layer is stratified. Although periclinal divisions were observed frequently in cells of the second layer, it was not possible to detect whether or not the frequency of such periclinal divisions was correlated with certain phases of apical growth.

Appearance of central zone, peripheral meristem and rib meristem: A central zone and peripheral and rib meristems can be recognized in an apex based on the appearance of cell protoplasts. This cytohistologic zonation is superimposed on the tunica-corporis pattern. As already explained in the first paper (Hara, 1971a) the central zone, which may correspond to the metrameristem (cf., Johnson and Tolbert, 1960), consists of relatively vacuolated, lightly stained, slowly dividing cells, while the peripheral meristem consists of relatively small, densely stained, actively dividing cells. The rib meristem, which is intimately concerned with pith development, occurs directly below the central zone and is surrounded by the peripheral meristem.

The width of the central zone is smaller in the minimal area phase than in the maximal area phase. The number of cells in the width varies slightly with the direction of sectioning of the apex, but the depth of the central zone, consisting of approximately 5 or 6 cells, scarcely changes during a plastochron.

Initiation of leaf primordium: When plastochronic changes of an apex having helically arranged leaf primordia are studied in longisections, it should be remembered that one obtains a median section of a leaf primordium or sector in a median section of the apex only by chance.

It is well known that the first evidence of initiation of a leaf primordium in a dicotyledonous shoot apex is commonly the occurrence of periclinal cell divisions in the second or a deeper cell layer; these cell divisions do not occur in the central zone but in the peripheral meristem near it. As explained earlier (Hara, 1971a), however, activity associated with leaf initiation in the first cell layer starts prior to the beginning of sector 0. Anticlinal cell divisions, which show the starting of this activity, can be observed readily in peripheral zone 4 near the base of the leaf during the maximal area phase. This is shown in the first cell layer (Fig. 1). Cell division activity subsequently spreads toward the central zone, and reaches there during the next plastochron. Anticlinal cell divisions also occur in the second or deeper cell layers (Figs. 2-4).

For *Clethra barbinervis* the periclinal cell divisions which mark leaf initiation in the second cell layer are correlated with the anticlinal cell divisions which mark sector initiation in the first cell layer. Certainly the periclinal divisions are observed more or less close to the base of leaf 5 in sector 0 during the minimal area phase (Fig. 2), and

Figs. 1-4. Median longisections of apex, including roughly-median section of a sector. ($\times 182$) Fig. 1: sector 4 in maximal area phase of apex; Fig. 2: sector 0 in minimal area phase; Fig. 3: sector 0 in maximal area phase; Fig. 4: sector 1 in minimal area phase. Arrows show sectors.

such divisions spread toward the central zone (Fig. 3). From this time, sector growth enters roughly the stage of sector 0 in the middle or maximal area phase of the apex. Elevation of a leaf primordium actually occurs during the next minimal area phase (Fig. 4).

Summary Analysis and Discussion

Since the form of the vegetative shoot apex is generally more or less convex, it is very difficult to analyze its three-dimensional construction, and there have been only a few studies in which apices were analyzed from the three-dimensional viewpoint.

If the apex is perfectly flat, the first transection may include the total area of the apex. The apex of *Daphne pseudo-mezereum*, studied by Hara (1962), is moderately flat; that of *Clethra barbinervis*, also not strictly flat, is flatter than the former, however. When the sections are 10μ thick, the apex of *Clethra* above the axil of the fifth leaf primordium normally can be included in 3 or 4 successive transections. As already reported (Hara, 1971 a, b), the flat apex allows analysis of superficial and transectional constructions of the apex. Consequently it also allows analysis for correlation between longisectional, superficial and transectional constructions.

There have been various studies in which the apical region and its underlying derivatives were correlated (Bartels, 1960; Steffensen, 1968; Neilson-Jones, 1969; Stewart and Dermen, 1970). Analysis for derivation of various tissues from the apex

Figs. 5-7. Figures of various zones of apex drawn from reconstructed figures of first cell layer of apices in superficial view. Fig. 5: minimal area phase, drawn from Fig. 15 of first paper of present series (Hara, 1971a); Fig. 6: middle area phase, drawn from Fig. 18 of first paper (inverted); Fig. 7: maximal area phase, drawn from Fig. 21 of first paper. 0: sector 0; 1-4: leaves 1-4; c: central zone; areas between c and 1, 2, 3, or 4: peripheral zones 1, 2, 3 and 4, respectively.

Figs. 8-10. Overlapped reconstructed figures of first cell layer of apices, showing correlations between different stages of same zone. Fig. 8: Fig. 5 was drawn in broken lines over Fig. 7; Fig. 9: Fig. 6 in broken lines over Fig. 5; Fig. 10: Fig. 7 in broken lines over Fig. 6. 0-4, c: same as for Figs. 5-7.

was not the primary intended of the present study; rather the three-dimensional construction of the apex is analyzed.

Analysis for overlapping of reconstructed figures of the first cell layer of the apex: Figs. 5, 6 and 7 are superficial views of an apex in the minimal, middle and maximal area phases respectively, showing the various areas of the apex. Fig. 8 was drawn by superimposing Fig. 5, using broken lines, over Fig. 7, Fig. 9 by Fig. 6 over Fig. 5, and Fig. 10 by Fig. 7 over Fig. 6. Figs. 5, 6 and 7 are the same outlines as Figs. 15, 18 and 21 in the first paper (Hara 1971a); although Fig. 6 was inverted. The apices drawn in these figures have the median value of three apices for the area of the apex in each of the three phases (cf., Hara, 1971a, b). Comparisons between apices in different area phases can thus be made, although the apices now compared are, of course, from different shoots. It is impossible in overlapping two figures to find a single corresponding point of the two. Thus, when two figures were overlapped, as in Figs. 8, 9 and 10, the central point of the central zone in each figure was not treated as a corresponding point, but balances between positions of sets of corresponding leaf primordia in the two figures were considered primarily in deciding the position of the overlap.

If the overlapped figures (Figs. 8–10) are considered in conjunction with the quantitative measurements given in the second paper (Hara, 1971b), developmental changes occurring during a plastochron are more clearly understood. It could not be decided, however, whether or not the center of the apex moves slightly during a plastochron, although Soma and Ball (1963) and Newman (1965) have suggested it as an important question. Changes in both size of the central zone and relative positions of sectors during a plastochron are remarkable. The position changes are particularly interesting. For example, between the maximal area phase of the apex (solid lines) and the minimal (broken lines) in Fig. 8, leaf 1 actively grows, using up the area which was formerly the peripheral zone, and becomes leaf 2. Also, a new peripheral zone is formed in front of leaf 2 of minimal area phase (broken lines in Fig. 8, solid lines in Fig. 9) using up the area which was formerly part of the central zone. Subsequently this leaf primordium (leaf 2) is pushed outward by active expansion of the apex (Fig. 9). Active growth of the peripheral zone begins after this stage. Although some parts of the peripheral zone are lost by being incorporated in successively initiating sectors and by expansion of the subtending leaf primordium, peripheral zone 4 of the maximal area phase (broken lines in Fig. 10, solid lines in Fig. 8) grows to become the center of sector 0 in the subsequent minimal area phase of the apex (Fig. 8). Thus, the interpretation of correlations between the central zone and various sectors presented in the earlier papers (Hara, 1971a, b) is substantiated by analysis of the overlapped figures (Figs. 8–10).

Consideration of dorsiventrality of the leaf: As has been mentioned already, activity leading to formation of a sector or of a leaf primordium begins near the base of an older leaf primordium. Thus, formation of a leaf begins on its abaxial side, and, at least during the earlier stages of sector development, the adaxial part of a primordium is build up by cells supplied from the peripheral zone, which, in turn, is derived from

Figs. 11-19. T divisions analysis of reconstructed figures of first cell layer of apices shown in Figs. 14-22 of first paper (Hara, 1971a). Figs. 11-13: minimal area phase; Figs. 14-16: middle area phase; Figs. 17-19: maximal area phase. 0-4, c: same as for Figs. 5-7.

the central zone. This means that initiation and elevation of the leaf primordium are earlier in the abaxial than in the adaxial part of the primordium. This, perhaps, may be one of factors causing the leaf to show dorsiventrality.

It is not certain from the present study whether or not cells at the tip of the mature leaf are derivatives of the cells elevated originally from the shoot apex at the time of its initiation.

Analysis of T divisions in the apex: Except within the central zone, an increase in files of cells constituting the peripheral meristem normally occurs in superficial and transectional views of the apex. Schüepp's Körper-Kappe analysis for the root

Figs. 20-23. T divisions analysis of median longisections of the apices shown in Figs. 1-4.

apex (Schüepf, 1917) is based on orientation of T divisions (cf., Clowes, 1961). As explained by Esau (1965), the combination of transverse and longitudinal divisions results in an approximately T- (or Y-) shaped wall pattern in the root apical meristem; such divisions with resulted files of cells have been named T divisions. T divisions are not restricted to the root apex. They can be seen when reconstructed figures of the first cell layer of apices are examined; there the combination of divisions in a plane perpendicular to files of cells and those in a plane parallel to the files results in an approximately T-shaped wall pattern. Analyses for this wall pattern, here called "T divisions analysis," are shown in Figs. 11-19 for the reconstructed figures of the first

cell layer of the apex given in the first paper (Hara, 1971a). T divisions were drawn when two or more successive cells showed divisions in a plane parallel to files of cells and were not drawn when only a single cell had divided.

It is clear that the number of T divisions in the peripheral zone increases from sector 1 to sector 4, reaching a maximum at the area of sector 0 in the maximal area phase. At the beginning of leaf 1 a number of T divisions occur within the protoderm of the primordium (leaf 1, Figs. 11, 12, 15). More top strokes of the T divisions face the central zone, although some do face the leaf primordium. T divisions which face the leaf primordium, however, are paired with other T divisions which face the central zone. In some instances a pair of T divisions may become connected with another basal T division facing the central zone by divisions of intermediate cells and thus come to form a single T division.

Files of cells showing T divisions are readily detectable if they were recently formed. T divisions may become disturbed, however, with the lapse of time by readjustments in cell arrangement. Thus, it can be said that an area showing many T divisions is one in which the formation of new files of cells has just been vigorous. Although both of the active increase in cell number in a file of cells and of the active increase in number of files are necessary prior to leaf initiation at its presumptive site of the apex, there

Fig. 24. Diagram showing a three-dimensional reconstruction of half an apex; the apex is cut through its center and the mid region of sector 0. 0, 1, 3, 4, c: same as for Figs. 5-7; 5, 6: leaves 5, 6.

may be a slight time lag from the peak of the former increase to that of the latter. Increase in cell number in a file results from cell divisions in a plane perpendicular to the files, while increase in number of files follows cell divisions in a plane parallel to the files of cells. Checking for the latter increase is T divisions analysis.

Even in the case of longisections T divisions analysis can be applied, as shown in Figs. 20–23 which are based on Figs. 1–4. The first indication of leaf initiation is more clearly recognized in an earlier stage of a sector growth than in the stage commonly accepted, as previously pointed out. A T division or a set of T divisions in the second or third layer in longisectional view appears in sector 0 at a point distant from the central zone in nearly the minimal area phase of the apex (Fig. 21) and spreads to the base of the new leaf primordium until the next minimal area phase (Figs. 22, 23). Thus, T divisions analysis applied to both longisectional and superficial views helps our understanding of the three-dimensional construction of a shoot apex. Fig. 24 is a diagram which tentatively expresses the three-dimensional construction of the vegetative shoot apex of *Clethra barbinervis*.

It is possible that T divisions analysis can be applied to both procambium and pith development, although it was not tried in the present study. In various studies of the vascular cambium (cf., Philipson and Ward, 1965) the analysis for increase and decrease in cell rows in the secondary xylem and phloem, which are derived from the cambium, is basically a T divisions analysis.

Observations and discussion in the present study were restricted to the extreme apical regions of the vegetative shoot apex. Although I was not successful in applying T divisions analysis to the second and deeper layers of the apex in transectional view, I believe that such analysis is needed for complete understanding of the three-dimensional construction of the apex.

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